Lead and eagles: demographic and pathological characteristics of poisoning, and exposure levels associated with other causes of mortality

J. Christian Franson · Robin E. Russell

Abstract We conducted a retrospective analysis to evaluate demographic and pathologic characteristics in 484 bald eagles (*Haliaeetus leucocephalus*) and 68 golden eagles (*Aquila chrysaetos*) diagnosed with lead poisoning at the U.S. Geological Survey National Wildlife Health Center. As part of our analysis, we compared characteristics of lead poisoned eagles with those that died of other causes. Odds of lead poisoning were greater for bald eagles versus golden eagles, females versus males, adults versus juveniles, and eagles from the Mississippi and Central flyways versus the Atlantic and Pacific flyways. In addition to spatial, species, and demographic associations, we detected a distinct temporal trend in the collection date of lead poisoned bald eagle carcasses. These carcasses were found at greater frequency in late autumn and winter than spring and summer. Lesions in lead poisoned birds included emaciation, evidence of bile stasis, myocardial degeneration and necrosis, and renal tubular nephrosis and necrosis. Ingested lead ammunition or fragments were found in 14.2 % of bald eagles and 11.8 % of golden eagles. The overall mean liver lead concentration (wet weight basis) for eagles diagnosed with lead poisoning was 28.9 ± 0.69 SE mg/kg in bald eagles and 19.4 ± 1.84 SE mg/kg in golden eagles. In eagles diagnosed with collision trauma, electrocution, poisoning (other than lead), emaciation, infectious disease, trapping death, other, and undetermined causes, average liver lead concentrations were low (<1 mg/kg) and did not differ among causes of mortality. Thus, based on our data, we found no evidence that lead exposure of eagles predisposed them to other causes of mortality.

Keywords Bald eagle · Golden eagle · Lead exposure · Lead poisoning · Mortality · Pathology

Introduction

Large birds of prey with opportunistic food habits, the bald eagle (*Haliaeetus leucocephalus*) is an exclusively North American species whereas the distribution of the golden eagle (*Aquila chrysaetos*) includes a wider area of the northern hemisphere. In North America, preferred foods are live fish for bald eagles and small to medium sized mammals for golden eagles, but both species will feed on carrion, particularly in the winter (Buehler 2000; Kochert et al. 2002). The bald eagle has a broad breeding range and winters primarily in the contiguous U.S. and coastal Canada and Alaska and, although golden eagles inhabit a wide range of latitudes in North America, they occur predominantly in the West (Buehler 2000; Kochert et al. 2002). Bald eagles are typically found near lakes, streams, and shorelines, whereas golden eagles frequent open terrestrial habitats. Although bald eagles were classified as endangered in the U.S. in 1967, populations have recovered considerably and they were removed from the endangered species list in 2007 (DOI 2007). Less is known about golden eagle populations, and various studies have described their numbers in the western U.S. as increasing, stable, or declining depending on location and whether nesting or migration counts were evaluated (Kochert and Steenhof 2002; Kochert et al. 2002; Smith et al. 2008).

In North America, mortality of birds due to lead poisoning from the ingestion of lead shotgun pellets was first

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reported in waterfowl in 1894 in Texas and North Carolina, and by the 1950s an estimated 2–3 % (1.6–2.4 million) of waterfowl across all North American flyways were dying annually of lead shot poisoning (Grinnell 1894; Hough 1894; Bellrose 1959). The first published report of lead poisoning in a bald eagle was in an emaciated bird found in Maryland in 1968 with two ingested lead shot (Mulhern et al. 1970). Additional reports followed, and lead poisoning of bald eagles was linked to the ingestion of lead shot embedded in tissues of unretrieved waterfowl shot by hunters and, rarely, in the gastrointestinal tract of waterfowl that themselves died of lead poisoning (Kaiser et al. 1980; Pattee and Hennes 1983; Reichel et al. 1984). Fluoroscopy of waterfowl for embedded shot and food habits studies of wintering bald eagles, using regurgitated casts, provided additional evidence of the association of lead poisoning in bald eagles and their consumption of waterfowl (Griffin et al. 1980; Sabine and Klimstra 1985).

In the U.S., continued poisoning of bald eagles and waterfowl from ingested shot led to a nationwide ban on the use of lead shot for hunting waterfowl and American coots (Fulica americana) in 1991 (Friend et al. 2009). However, lead shotgun pellets and rifle bullets are still widely used for hunting upland and large game animals and the possibility of lead poisoning from the ingestion of lead ammunition and fragments thereof persists in eagles in North America. For example, in a study of bald and golden eagles admitted to a rehabilitation center between 1980 and 1995, the prevalence of lead poisoning did not change after the 1991 implementation of nontoxic shot regulations for waterfowl, although the mean blood lead concentration decreased (Kramer and Redig 1997). A follow-up study indicated that the frequency of elevated blood lead concentrations in bald eagles increased an average of 5 % per year from 1996 through 2009 (Cruz-Martinez et al. 2012). In addition, both reports found that admissions of lead exposed eagles to the rehabilitation center coincided with deer (Odocoileus sp.) hunting seasons, suggesting an association between lead poisoning in eagles and deer offal left in the field. Large numbers of lead fragments have been found in deer killed with lead rifle ammunition and recent reports have provided additional evidence linking lead exposure of eagles with discarded carcasses and offal (Hunt et al. 2006; Neumann 2009; Bedrosian et al. 2012; Cruz-Martinez et al. 2012; Warner et al. 2014).

Lead is a cumulative metabolic poison that affects all body systems and causes a variety of adverse effects, including neurological, behavioral, developmental, and immunological deficits (Hunter and Wobeser 1980; Eisler 2000; Franson and Pain 2011). For example, common terns (Sterna hirundo) and American herring gulls (Larus smithsonianus) dosed with lead exhibited impairments of locomotion, balance, righting behavior, and depth perception (Burger 1995). These and other findings raise the question of whether lead exposure in birds may be a predisposing factor for causes of mortality such as collision with objects, including power lines resulting in electrocution, disease, and predation. Some evidence exists to support this idea. Bellrose (1959) reported that mallards (Anas platyrhynchos) dosed with two or four lead shot pellets and released were about twice as vulnerable to being killed by hunters as controls. Two studies of mute swans (Cygnus olor) reported an association between blood and tissue lead concentrations and collision with objects, including power lines (O'Halloran et al. 1989, Kelly and Kelly 2005).

A retrospective summary of causes of mortality in more than 4,400 bald eagles and golden eagles submitted to the National Wildlife Health Center (NWHC) from throughout the U.S. during 1975–2013 identified the same top four causes of death, although not in the same order, for both species: poisoning, trauma, electrocution, and shooting (Russell and Franson 2014). In that report, lead toxicosis was the most frequently diagnosed poisoning in both species, comprising 63.5 % of all poisonings in bald eagles and 58.1 % in golden eagles. As a proportion of the total number of bald eagles examined at the NWHC, the frequency of those diagnosed with lead poisoning increased in the U.S. after implementation of the 1991 restrictions on hunting waterfowl with lead shot (Russell and Franson 2014). The largest increase, from 6 % before 1991 to 30 % after 1991 occurred in the Pacific flyway (Russell and Franson 2014). No increase in lead poisoning was detected in golden eagles, but the sample size for that species was much smaller (Russell and Franson 2014). Submission of eagle carcasses to the NWHC was not based on randomized surveys, hence the increase in the frequency of lead poisonings diagnosed in bald eagles after 1991 may not necessarily reflect the true proportional change. However, we believe that lead exposure remains an important issue for both bald and golden eagles. In this report, we describe the demographic and pathologic characteristics of a large sample of eagles diagnosed with lead poisoning. To address the question of whether or not sublethal lead exposure may render eagles more susceptible to other causes of mortality, we also compared the liver lead concentrations among eagles diagnosed with causes of mortality other than lead poisoning.

Methods

Case records, necropsy, and lead analysis

The data used in our study came from case files of bald and golden eagles necropsied at the NWHC (Russell and Franson 2014). The time span for golden eagle carcass collection was from 1975 to 2013, but data for bald eagles were restricted to those collected from 1982 to 2013, as causes of mortality,

including lead poisoning, diagnosed in carcasses collected from 1975 to 1981 were reported by Kaiser et al. (1980) and Reichel et al. (1984). Necropsy procedures and preparation of tissues for histopathology were as previously described (Franson et al. 1996). Histologic sections of kidney tissue from a subset of eagles (13 bald eagles and 4 golden eagles) diagnosed with lead poisoning were stained with Ziehl Neelsen and Fite's acid-fast stains and examined for acid-fast intranuclear inclusion bodies in proximal tubular epithelial cells, an indication of lead poisoning (Franson and Pain 2011). Lead analysis of liver tissue was by atomic absorption spectroscopy (Haseltine et al. 1981; Franson and Smith 1999). The lower limit of detection (LOD) ranged from 0.1 to 0.25 mg lead per kg liver, wet weight. Average recovery of lead from 154 spiked samples was 98 %. All results are expressed on wet weight basis. Pathologists performing necropsies assigned a diagnosis of lead poisoning in individual eagles based on an evaluation of clinical signs (when reported), gross and microscopic lesions, and tissue lead concentrations as described by Locke and Thomas (1996) and Franson and Pain (2011). Typically, gross observations including emaciation and characteristics of bile stasis, microscopic lesions such as damage to the kidney and heart, in combination with liver lead concentrations of >6 mg/kg wet weight, support a diagnosis of lead poisoning (Locke and Thomas 1996; Franson and Pain 2011).

Statistical analysis

Characteristics of lead poisoned versus non-lead poisoned eagles

To identify the important factors associated with lead poisoning in eagles, we compared the ecological and demographic characteristics of lead poisoned eagles with eagles that died of other causes (Russell and Franson 2014). To accomplish this, we conducted a logistic regression using a generalized linear model to evaluate the relationship between age, sex, species, and flyway with the proportion of eagles diagnosed with lead poisoning (Hosmer and Lemeshow 2000). We compared candidate models using Akaike information criterion (AIC). Models within 2 AIC values of each other were considered equivalent (Burnham and Anderson 2002). We conducted the analyses using the R function 'glm' and specified a logit link (the appropriate specification for binomial data) (R Core Team 2014). We ran all one, two, and three factor combination models, the full model (all four variables) with and without interaction terms, and the null model (intercept only model) for a total of 25 models. The top model was evaluated using the Hosmer–Lemeshow goodness of fit test, and area under the receiver operating curve (Hosmer and Lemeshow 2000; Lasko et al. 2005).

We also evaluated our data for potential seasonal trends in lead poisoned eagle carcass collections. To evaluate temporal distributions in the proportion of eagles diagnosed as lead poisoned, we used a χ^2 analysis to compare proportions of lead poisoned and non-lead poisoned eagles collected in each month of the year. To evaluate the differences in body mass between lead poisoned eagles and those diagnosed with all other causes of mortality, including emaciation (Russell and Franson 2014), we conducted a linear regression analysis with body mass as the response variable. We included the covariates lead poisoned versus non-lead poisoned, species, age, and sex in our candidate models. We conducted a linear regression with covariates of lead poisoned versus non-lead poisoned, species, age, and sex to assess the important factors associated with body mass. We ran all one, two, three factor models, the full model (all four variables) with and without interaction terms, and the null model (intercept only model) for a total of 25 models. We ran the analyses using the function 'glm' in R with a "Gaussian" link function, the appropriate function for normally distributed data (R Core Team 2014). As with the previous logistic regression analysis, we compared candidate models using AIC.

Ingested lead and liver lead concentrations

To determine the most important factors associated with lead liver concentrations in lead poisoned eagles we ran several candidate models with age, sex, species of eagle, and presence or absence of ingested lead. We ran the analyses using the function 'glm' in R with a "Gaussian" link function, the appropriate function for normally distributed data (R Core Team 2014). We compared candidate models using AIC. We ran all one, two, and three factor models, the full model (all

Table 1 Sample sizes for eagles by species, age, and sex for (a) lead poisoned eagles (484 bald eagles and 68 golden eagles (Yes = ingested lead; No = no ingested lead) and (b) non-lead poisoned eagles (1,733 bald eagles and 491 golden eagles)

(a)	Female				Male			
	Adult		Juvenile		Adult		Juvenile	
	Yes	No	Yes	No	Yes	No	Yes	No
Bald eagle[a]	37	182	13	66	12	129	4	30
Golden eagle[b]	2	24	1	18	2	12	3	2

(b)	Female		Male	
	Adult	Juvenile	Adult	Juvenile
Bald eagle[c]	436	450	426	373
Golden eagle[d]	51	133	40	231

[a] 11 Bald eagles of unknown age and/or sex (3 with ingested lead)

[b] 4 Golden eagles of unknown age and/or sex (2 with ingested lead)

[c] 48 Bald eagles of unknown age and/or sex

[d] 36 Golden eagles of unknown age and/or sex

Table 2 Top five models (of 25 examined) including the full model, and the null model for the odds of lead poisoning for bald and golden eagle carcasses submitted to the NWHC (lead poisoned birds of known age and sex: 473 bald eagles and 64 golden eagles; non-lead poisoned birds of known age and sex: 1,685 bald eagles and 455 golden eagles)

	df	AIC	ΔAIC	AICwt
Full model, no interactions	7	2,824.79	0.00	0.97
Full model, all interactions	32	2,831.47	6.68	0.03
Age, species, flyway, all interactions	16	2,841.89	17.10	0.00
Age, species, flyway, no interactions	6	2,843.88	19.09	0.00
Age, sex, flyway, all interactions	16	2,893.56	68.76	0.00
Null model	1	3,175.17	350.38	0.00

The lowest AIC value indicates the best model in the model suite

AIC Akaike information criterion, *ΔAIC* the difference between the model and the model in the set with the lowest AIC value, *AICwt* Akaike information criterion weight

four variables) with and without interaction terms, and the null model (intercept only model) for a total of 25 models. We assessed model fit with R-squared values.

We used analysis of variance (ANOVA) to estimate and compare liver lead concentrations among eagles diagnosed with collision trauma, electrocution, poisoning (other than lead), emaciation, infectious disease, trapped, gunshot, other, and undetermined. We conducted the analysis in a Bayesian framework to account for observed lead levels below the lower limits of detection (R2WinBUGS, Sturtz et al. 2005). Lead liver concentrations (y) were modelled as normally distributed variables with mean (μ) and precision (τ) (i.e. $y_i \sim N(\mu_i, \tau)$); where precision is 1/variance (see Kéry 2010 for example code). We then used the standard ANOVA analyses $\mu_i = \alpha_s$ where α is an intercept term and s is the subgroup, in our case the cause of death (the number of α will equal the number of subgroups). Lead concentrations below the LOD of 0.10 or 0.25 (n = 1,178) were recorded as missing (NA) and estimated in WinBUGS using a censored distribution (0, LOD); specifically $y_i \sim N(\mu_i, \tau)I(0, LOD)$ (Route et al. 2014). We ran three chains for 40,000 iterations after a burn-in period of 10,000. Convergence was assessed by visual inspection of the chains and R-hat values (with R-hat values ∼1 indicating convergence).

Results

Characteristics of lead poisoned versus non-lead poisoned eagles

Lead poisoned bald eagles (n = 484) and golden eagles (n = 68) (Table 1) were submitted from 38 and 20 states, respectively. Seven states (AK, AR, IA, FL, MN, MO, and
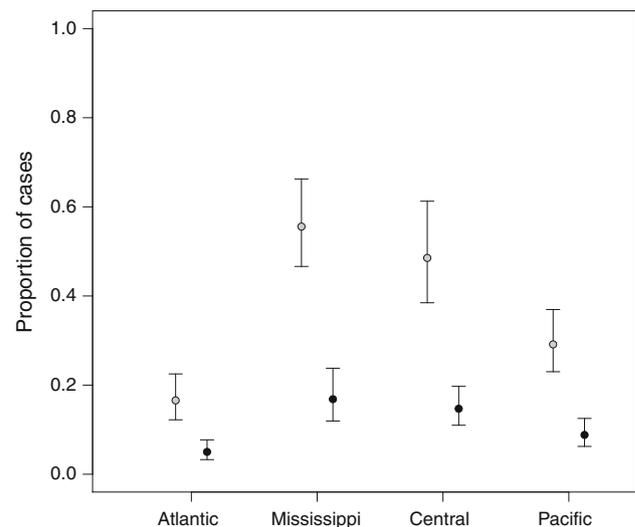

Fig. 1 The predicted probability of lead poisoning for adult female bald (*light dots*) and golden (*black dots*) eagles by flyway. Estimates are derived from data on lead poisoned eagles (484 bald eagles and 68 golden eagles) and non-lead poisoned eagles of known age and sex (2,315 bald eagles and 1,197 golden eagles) submitted to the National Wildlife Health Center between 1975 and 2013. *Bars* represent 95 % Credible Intervals

WI) accounted for 51 % of poisoned bald eagles and 50 % of golden eagles came from four states (ID, MT, OR, SD). The best model for estimating the odds of lead poisoning in eagles was one containing, age, sex, species, and flyway (Table 2). The Hosmer–Lemeshow goodness of fit test indicated an adequate fit to the data ($\chi^2 = 10.0$, df = 8, p = 0.261). No other model was within 2 AIC values of this top model. The area under the receiver operating curve was 0.74 (i.e., random draws from the lead poisoned and non-lead poisoned eagle data sets would result in correct predictions 74 % of the time).

Results from the best model indicated that odds of lead poisoning were higher for bald eagles than golden eagles (odds ratio = 3.25, 95 % Credible Interval (CI) 2.43-4.43), females versus males (odds ratio = 1.57, 95 % CI 1.29–1.91), and adult versus juveniles (odds ratio = 3.39, 95 % CI 2.76–4.22). Odds also differed by region with the Central and Mississippi flyways having the largest proportion of lead poisoned eagles. For example, the estimates from the model for adult female eagles by flyway are shown in Fig. 1. Chi square analysis indicated that the proportion of lead poisoned versus non-lead poisoned bald eagles differed by month ($\chi^2 = 58.9$, df = 11, p < 0.0001), but no such difference was noted for golden eagles ($\chi^2 = 8.8$, df = 11, p = 0.644) (Fig. 2).

Body condition and pathology

More than half of the lead poisoned eagles were characterized as emaciated or in poor body condition (Table 3).

(a)

(b)
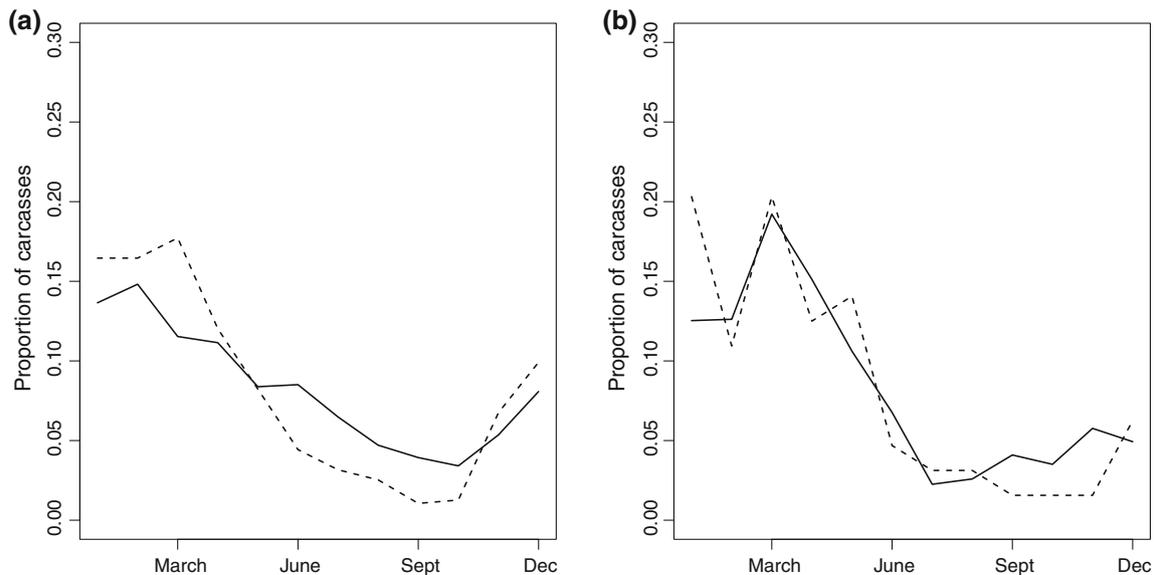
Fig. 2 The proportion of lead poisoned eagles versus non-lead poisoned eagles with known collection date, collected by month and submitted to the National Wildlife Health Center 1975–2013. **a** bald eagles (484 lead poisoned and 2,315 non-lead poisoned), **b** golden eagles (68 lead poisoned and 1,197 non-lead poisoned). *Solid line* is non-lead poisoned eagles, *dotted line* is lead poisoned eagles

Table 3 Frequency (%) of gross and microscopic lesions observed in lead poisoned bald eagles (n = 484) and golden eagles (n = 68)

	Bald eagle	Golden eagle
Gross lesions		
Enlarged gall bladder	61.2	67.6
Bile staining, GI tract[a]	21.9	35.3
Bile staining, feathers[b]	6.2	4.4
Liver atrophy	27.3	39.7
Body condition		
Emaciated/poor	53.1	67.6
Fair/good	40.1	20.6
Excellent	4.8	4.4
Not described	1.6	7.4
Microscopic lesions[c]		
Myocardial degeneration/necrosis	36.4	42.3
Renal tubular nephrosis/necrosis[d]	5.4	17.6

[a] Bile staining anywhere in gastrointestinal tract, but primarily in stomach and/or intestine

[b] Bile staining on feathers around vent or mouth

[c] Heart (n = 299 for bald eagles, 26 for golden eagles). Kidney (n = 182 for bald eagles, 17 for golden eagles)

[d] No acid-fast intranuclear inclusion bodies were observed in kidney sections

The best model of body mass included the variables sex, age, lead poisoned versus non-lead poisoned, and all interaction terms (Table 4). R-squared values were 0.25. The body mass of lead poisoned eagles was on average lower than that of non-lead poisoned eagles, with estimated differences of 782 g for adult female eagles, 802 g for

Table 4 Top five models (of 25 examined), including the full model, and null model, relating age, species, sex, and lead poisoning to body mass (lead poisoned birds of known body mass: 455 bald eagles and 61 golden eagles; non-lead poisoned birds of known body mass: 1,689 bald eagles and 456 golden eagles) for bald and golden eagle carcasses submitted to the NWHC

	df	AIC	ΔAIC	AICwt
Age, sex, lead poisoned, all interactions	9	43,347.84	0.00	0.91
Full model, all interactions	17	43,353.01	5.17	0.07
Age, sex, lead poisoned	5	43,355.66	7.82	0.02
Full model, no interactions	6	43,357.40	9.56	0.01
Sex, species, lead poisoned	5	43,446.50	98.66	0.00
Null model	2	44,125.95	778.11	0.00

The lowest AIC value indicates the best model in the model suite

AIC Akaike information criterion, *ΔAIC* the difference between the model and the model in the set with the lowest AIC value, *AICwt* Akaike information criterion weight

adult males, 620 g for juvenile females, and 655 g for juvenile males. Additional gross lesions included enlarged gall bladder, bile staining of the gastrointestinal tract and feathers, and liver atrophy (Table 3). The histopathologic lesions described included myocardial degeneration and necrosis, as well as renal tubular nephrosis and necrosis (Table 3). No acid-fast intranuclear inclusion bodies were seen in renal tubular epithelial cells of kidney sections from the 17 eagles examined. Mean liver lead concentrations in those 17 birds were 27.1 mg/kg in bald eagles and 11.4 mg/kg in golden eagles.

Table 5 Top five models (of 25 examined), the full model and null model, relating age, species, sex, and ingested lead to liver lead concentrations for bald and golden eagle carcasses submitted to the NWHC and diagnosed with lead poisoning

	df	AIC	ΔAIC	AICwt
Age, species, ingested lead	5	4,337.45	0.00	0.67
Age, species, ingested lead, sex	6	4,339.45	2.00	0.25
Species, ingested lead	4	4,342.91	5.46	0.04
Age, species, ingested lead, all interactions	9	4,344.53	7.09	0.02
Species, ingested lead, all interactions	5	4,344.73	7.29	0.02
Full model, all interactions	17	4,351.634	14.185	0.00
Null model	2	4,404.79	67.341	0.00

The lowest AIC value indicates the best model in the model suite

AIC Akaike information criterion, *ΔAIC* the difference between the model and the model in the set with the lowest AIC value, *AICwt* Akaike information criterion weight

Table 6 Liver lead concentrations (mg/kg wet weight) in bald (n = 482 of known age) and golden eagles (n = 67 of known age) diagnosed with lead poisoning by species, age, and presence or absence of ingested lead

Species	Age[a]	Ingested lead (n)	Mean	SE
Golden eagle	Juvenile	No (22)	13.37	1.95
		Yes (4)	23.08	2.42
	Adult	No (36)	17.08	1.84
		Yes (5)	26.85	2.51
Bald eagle	Juvenile	No (96)	24.12	1.30
		Yes (19)	33.89	2.12
	Adult	No (317)	27.82	0.90
		Yes (50)	37.52	1.69

[a] Excludes one golden eagle and two bald eagles of unknown age

Ingested lead and liver lead concentrations

In lead poisoned birds, ingested lead ammunition or fragments were noted in 14.2 % of bald eagles and 11.8 % of golden eagles. These were described as lead shotgun pellets (50.6 %), lead bullets and fragments (45.4 %), and bullet jackets (3.4 %). The overall mean concentration of lead in livers of poisoned eagles was 28.9 mg/kg wet weight for bald eagles and 19.4 mg/kg wet weight for golden eagles. In 99.3 % of bald eagles and 94.1 % of golden eagles diagnosed with lead poisoning, liver lead concentrations were ≥6 mg/kg.
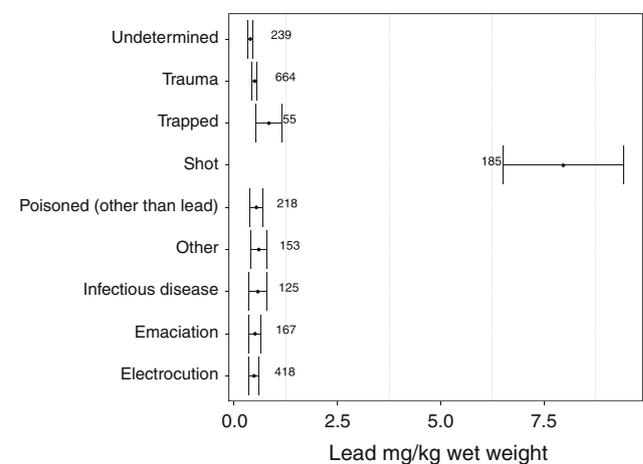
Linear regression results indicated that best models of liver lead concentrations in lead poisoned eagles included the covariates age, species, and ingested lead (Table 5). The second best model included the covariate sex as well, however, this parameter estimate was not statistically significant (coefficient estimate = 0.07, SE = 1.24, *t* value = 0.058, *p* value = 0.9535). Therefore, we interpreted the model with age, species and ingested lead only. The parameter estimates indicated that, on average, liver lead concentrations were 10.74 mg/kg (95 % CI 7.16–14.32) higher in bald eagles than golden eagles, 3.7 mg/kg (95 % CI 1.05–6.38) higher in adult eagles than juveniles, and 9.70 mg/kg (95 % CI 6.34–13.04) higher in eagles with ingested lead than in those without ingested lead (Table 6). However, the overall fit of the model was poor (R-squared ∼0.13).

With the exception of shooting, our ANOVA analysis revealed no differences in liver lead levels among other causes of mortality diagnosed in eagles, and estimated liver lead concentrations for all other causes of death were <1 mg/kg wet weight (Fig. 3). Lead concentrations in

Fig. 3 Mean (95 % Credible Interval, n) liver lead concentration in 2,224 bald and golden eagles diagnosed with causes of mortality other than lead poisoning

livers of gunshot eagles were estimated as 7.97 mg/kg wet weight (95 % CI 6.50–9.43). Liver lead concentrations of 98, 108, and 1,100 mg/kg in three shot eagles (two bald, and one golden) contributed to this estimate.

Discussion

Scavenging of unretrieved carcasses or offal left in the field from animals other than waterfowl killed or wounded with lead ammunition has been suggested as the primary pathway for continued lead exposure and poisoning in eagles (Wayland et al. 2003; Neumann 2009; Pain et al. 2009; Stauber et al. 2010; Bedrosian et al. 2012; Nadjafzadeh et al. 2013). Some of these studies have reported greater lead exposure frequency in eagles in autumn and winter months, linking exposure to time periods during and after

hunting seasons. Our data also indicated a tendency for lead poisoned bald eagles to be found more frequently from late autumn through the winter months, but less frequently during the summer, than bald eagles that died of other causes. Geographically, lead poisoned eagles were more likely to come from the Central and Mississippi flyways than from the Atlantic or Pacific. Possible explanations for this regional difference include a greater frequency of scavenging on carrion containing lead ammunition, greater frequency of animals shot with lead ammunition, and more carcasses or offal left in the field. Our finding that bald eagles were lead poisoned more frequently than golden eagles could be the result of golden eagles being more successful predators and less likely to feed on carrion containing lead fragments, or bald eagle carcasses being noticed more frequently, because of the white plumage on adults, than golden eagle carcasses. Displacement of golden eagles scavenging on lead-containing carcasses by bald eagles seems unlikely because of the aggressive nature of golden eagles (Halley and Gjershaug 1998). We also found that female eagles were diagnosed with lead poisoning more frequently that males and adults more frequently than juveniles. This may be at least partially explained by intraspecific dominance at scavenged carcasses, although there is more evidence for females displacing males than for adults displacing juveniles (Halley and Gjershaug 1998; Garcelon 1990). In combined data from two reports of lead poisoning in bald eagles, 18 of 26 birds were females and 14 of 26 were adults (Kaiser et al. 1980; Reichel et al. 1984).

Acute lead poisoning of birds is generally characterized by a high level of lead exposure for a short time, whereas chronic poisoning occurs over a longer period of time, often with a lesser magnitude of exposure (Franson and Pain 2011). Thus, birds dying of acute lead poisoning may be in good flesh with abundant fat reserves, but those chronically poisoned typically exhibit anorexia, leading to debilitation with loss of muscle mass and fat reserves (Locke and Thomas 1996). In our study, lead poisoned bald and golden eagles were considerably lighter than non-lead poisoned eagles and more than half were characterized as being in emaciated or poor body condition, suggesting that most were undergoing chronic poisoning and not obtaining adequate nutrition. Emaciation was the major gross lesion reported in a study of bald eagles dosed with lead shot (Pattee et al. 1981). Liver atrophy, a typical observation in emaciated birds, was noted in lead poisoned bald and golden eagles and has earlier been reported in sea eagles dying of lead poisoning (Fournier and Hines 1994; Saito 2009). In both bald and golden eagles, we also found evidence of bile stasis including enlarged gall bladder, bile staining of the gastrointestinal tract, and to a lesser extent bile staining of feathers, commonly reported findings in

lead poisoned eagles and other raptors (Kaiser et al. 1980; Reichel et al. 1984; Locke and Thomas 1996).

Microscopic lesions noted in our study included myocardial degeneration or necrosis and renal tubular nephrosis or necrosis, lesions previously reported in bald eagles with lead poisoning (Pattee et al. 1981). However, acid-fast intranuclear inclusion bodies were not seen in histologic sections of kidney tissue. Such inclusion bodies were reported in one free-ranging white-tailed eagle (*Haliaeetus albicilla*) with elevated tissue lead, but were not observed in a wild bald eagle diagnosed with lead poisoning or in an experimental study of lead shot poisoning in bald eagles (Jacobson et al. 1977; Pattee et al. 1981; Kenntner et al. 2001). Other raptors reported with lead-induced acid-fast renal inclusion bodies include turkey vulture (*Cathartes aura*), Andean condor (*Vulture gryphus*), eastern screech-owl (*Megascops asio*), snowy owl (*Bubo scandiacus*), and laggar falcon (*Falco jugger*) (Locke et al. 1969; Mac-Donald et al. 1983; Beyer et al. 1988; Carpenter et al. 2003).

Few lead poisoned eagles in our study (14.2 % of bald and 11.8 % of golden eagles) had lead fragments in their stomachs. Cruz-Martinez et al. (2012) found radiographic evidence of metal fragments in stomachs of 11 % of 322 bald eagles with elevated blood lead concentrations (>0.2 µg/ml). Neumann (2009) reported that, of 59 bald eagles radiographed, not all of which were lead poisoned, 12 % had what was described as lead in digestive tracts. Higher frequencies of ingested lead have been reported in studies with smaller sample sizes. For example, among 16 white-tailed eagles with concentrations of lead (>20 µg/g dry weight) in liver or kidney considered by the authors to be evidence of lead poisoning, 25 % had lead ammunition in their gastrointestinal tracts (Helander et al. 2009). Ingested lead shot were reported in 23 % of 26 lead poisoned bald eagles (data combined from Kaiser et al. 1980 and Reichel et al. 1984).

Once an eagle ingests a lead fragment, it may be eroded in the stomach, regurgitated, passed through the gastrointestinal tract, or be retained until death. As with other raptors, eagles egest pellets, or casts, of indigestible material consumed with food items, and lead shot have been found in pellets of bald eagles in the wild (Duke et al. 1976; Griffin et al. 1980; Nelson et al. 1989). The elapsed time between consumption of a meal and pellet egestion, or meal to pellet interval, in captive bald eagles in one study was about 22 h (Duke et al. 1976). If wild eagles egest casts with similar frequency, some apparently retain lead in the stomach through more than one meal to pellet interval, demonstrated by the presence of ingested lead in a fraction of birds, and the fact that that the majority absorbed enough lead to be poisoned but were found without lead in their stomachs. This is supported by findings of an experimental

study of lead poisoning in bald eagles, as it was reported that the lead shot administered were regurgitated as soon as 12 h after dosage or retained as long as 48 days (Pattee et al. 1981). Another factor to consider regarding the retention of lead in the eagle stomach is that the adverse effects of increased lead absorption and poisoning result in anorexia, as evidenced by the loss of body mass in affected birds. Fewer meals consumed result in fewer casts and a greater likelihood that lead will remain in the stomach. The fact that we found higher liver lead concentrations in poisoned eagles with ingested lead versus those without is consistent with a decline in liver lead concentration after the lead is voided or eroded.

The mean liver lead concentration of lead poisoned bald eagles in our study (28.9 mg/kg wet weight) was very similar to that reported by Kaiser et al. (1980) (29.9 mg/kg wet weight) and Reichel et al. (1984) (27.9 mg/kg wet weight), and well above 10 mg/kg wet weight, the concentration suggested by Franson and Pain (2011) as an indication of severe clinical poisoning. Golden eagles diagnosed with lead poisoning had lower liver lead concentrations, but still >10 mg/kg wet weight. Adult bald and golden eagles diagnosed with lead poisoning had higher liver lead concentrations than juveniles, similar to the findings reported by Kaiser et al. (1980) and Reichel et al. (1984) for bald eagles (combined data from both studies, adults = 31.8 mg/kg wet weight, juveniles = 24.8 mg/kg wet weight). In two studies of bald and golden eagles, elevated lead concentrations (>0.2 μg/ml in blood or 6-8 μg/g in liver or kidney) were found more frequently in adult and subadult age classes combined (23 and 19.5 %) than in immature birds (7 % in each study) (Wayland and Bollinger 1999; Wayland et al. 2003). Cruz-Martinez et al. (2012) suggested that the higher frequency of elevated blood lead concentrations (>0.2 μg/ml) in adult versus immature and hatch year bald eagles in their study was the result of adults being more aggressive when scavenging carcasses, thus having the first opportunity to consume tissue around bullet wound sites. However, such aggressive behavior would not explain higher liver lead concentrations in adult eagles that died of lead poisoning. Perhaps juveniles die of lead poisoning more slowly than adults and, as the majority of eagles in our study did not have lead fragments in the stomach, the liver lead concentrations in most would decline with increased survival time.

Some previous reports provide evidence linking lead exposure in birds to traumatic deaths, such as collisions with power lines or other objects, and impaired immune response. For example, the mean liver lead concentration in mute swans dying from collisions with electric or telephone wires was 33 mg/kg wet weight, compared with 8 mg/kg wet weight in swans dying of other causes (O'Halloran et al. 1989). Kelly and Kelly (2005) reported

that a significantly greater proportion of mute swans that suffered collisions with power lines had blood lead concentrations that the authors defined as moderately elevated compared with non-collision birds. Helander et al. (2009) found elevated lead concentrations in tissues of several white-tailed eagles recovered under power lines or with other indications of trauma. Immunosuppressive effects, evidenced by reduced immunologic cell numbers and antibody titers were noted in experimental studies in mallards dosed with lead shot (Trust et al. 1990; Rocke and Samuel 1991). Wayland et al. (2003) found that bald eagles with toxicoses other than lead poisoning had kidney lead concentrations that were higher than in those diagnosed with injuries, disease, or emaciation. The other toxins were primarily organophosphorus and carbamate compounds and many bald eagles poisoned by these compounds were found in areas with high lead exposure. The authors suggested that, rather than lead increasing the susceptibility of eagles to the pesticides, it was possible that the birds were being exposed to both lead and pesticides through their diet (Wayland et al. 2003). Rodriguez-Ramos Fernandez et al. (2011) found no association between lead concentrations in tissues of Spanish imperial eagles (*Aquila adalberti*) and the causes of morbidity and mortality resulting in their admission to rehabilitation centers. In a study of several birds of prey, proportionally more birds with unknown cause of death or those diagnosed as gunshot had elevated tissue lead concentrations than did birds with other causes of death (Pain et al. 1995). In our study we found that, except for eagles that were diagnosed as gunshot, liver lead concentrations in non-lead poisoned eagles were low (<1 mg/kg wet weight) and there were no differences among those diagnosed with other causes of mortality. We suspect the higher liver lead concentrations in gunshot eagles were the result of lead fragments or residue from lead ammunition passing through or near the liver (Frank 1986).

Conclusions

Important factors associated with the diagnosis of lead poisoning in eagles in the USA included species, sex, age, and location. The temporal distribution of lead poisoned bald eagles, but not golden eagles, differed from that of other causes of mortality. Among poisoned birds, liver lead concentrations were three times higher in bald eagles and twice as high in golden eagles as the commonly recommended threshold for severe poisoning. We noted several of the lesions typically associated with lead poisoning in birds, but an absence of acid-fast inclusion bodies in renal tubules. Important factors affecting the liver lead concentration of poisoned eagles included species, age, and

ingested lead ammunition. Liver lead concentrations in eagles diagnosed as collision trauma, electrocution, poisoning exclusive of lead, emaciation, infectious disease, trapped, other, and undetermined were low and there were no differences among the various categories. Thus, we found no evidence that lead exposure predisposed eagles to these other causes of mortality.

Conflict of interest The authors declare that they have no conflict of interest.

References

Bedrosian B, Craighead D, Crandall R (2012) Lead exposure in bald eagles from big game hunting, the continental implications and successful mitigation efforts. PLoS ONE 7:e51978

Bellrose FC (1959) Lead poisoning as a mortality factor in waterfowl populations. Ill Nat Hist Surv Bull 27:235–288

Beyer WN, Spann JW, Sileo L, Franson JC (1988) Lead poisoning in six captive avian species. Arch Environ Contain Toxicol 17:121–130

Buehler DA (2000) Bald eagle (*Haliaeetus leucocephalus*). In: Poole A (ed) The birds of North America online. Cornell Lab of Ornithology, Ithaca. http://bna.birds.cornell.edu/bna/species/506. doi:10.2173/bna.506. Accessed 9 May 2014

Burger J (1995) A risk assessment for lead in birds. J Toxicol Environ Health 45:369–396

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Carpenter JW, Pattee OH, Fritts SH, Rattner BA, Wiemeyer SN, Royle JA, Smith MR (2003) Experimental lead poisoning in turkey vultures (*Cathartes aura*). J Wildl Dis 39:96–104

Cruz-Martinez L, Redig PT, Deen J (2012) Lead from spent ammunition: a source of exposure and poisoning in bald eagles. Hum Wildl Interact 6:94–104

Department of the Interior (DOI) (2007) Endangered and threatened wildlife and plants: removing the bald eagle in the lower 48 states from the list of endangered and threatened wildlife. Fed Reg 72:37346–37372

Duke GE, Evanson OA, Jegers A (1976) Meal to pellet intervals in 14 species of captive raptors. Comp Biochem Physiol 53A:1–6

Eisler R (2000) Handbook of chemical risk assessment: health hazards to humans, plants, and animals, vol 1. Metals. Lewis Publishers, Boca Raton

Fournier MA, Hines JE (1994) Effects of starvation on muscle and organ mass of king eiders *Somateria spectabilis* and the ecological and management implications. Wildfowl 45:188–197

Frank A (1986) Lead fragments in tissues from wild birds: a cause of misleading analytical results. Sci Total Environ 54:275–281

Franson JC, Pain DJ (2011) Lead in birds. In: Beyer WN, Meador JP (eds) Environmental contaminants in biota: interpreting tissue concentrations, 2nd edn. CRC Press, Boca Raton, pp 563–593

Franson JC, Smith MR (1999) Poisoning of wild birds from exposure to anticholinesterase compounds and lead: diagnostic methods and selected cases. Seminars Avian Exotic Pet Med 8:3–11

Franson JC, Thomas NJ, Smith MR, Robbins AH, Newman S, McCartin PC (1996) A retrospective study of postmortem findings in red-tailed hawks. J Raptor Res 30:7–14

Friend M, Franson JC, Anderson WL (2009) Biological and societal dimensions of lead poisoning in birds in the USA. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, pp 34–60

Garcelon DK (1990) Observations of aggressive interactions by bald eagles of known age and sex. Condor 92:532–534

Griffin CR, Baskett TS, Sparrowe RD (1980) Bald eagles and the management program at Swan Lake National Wildlife Refuge. Trans N Am Wildl Nat Resour Conf 45:252–262

Grinnell B (1894) Lead poisoning. For Stream 42:117–118

Halley DJ, Gjershaug JO (1998) Inter- and intra-specific dominance relationships and feeding behaviour of golden eagles *Aquila chrysaetos* and sea eagles *Haliaeetus albicilla* at carcasses. IBIS 140:295–301

Haseltine SD, Heinz GH, Reichel WL, Moore JF (1981) Organochlorine and metal residues in eggs of waterfowl nesting on islands in Lake Michigan off Door County, Wisconsin, 1977–78. Pesticides Monit J 15:90–97

Helander B, Axelsson J, Borg H, Holm K, Bignert A (2009) Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. Sci Total Environ 407:5555–5563

Hosmer DW, Lemeshow S (2000) Applied logistic regression, 2nd edn. Wiley, New York

Hough E (1894) Lead-poisoned ducks. For Stream 42:117

Hunt WG, Burnham W, Parish CN, Burnham KK, Mutch B, Oaks JL (2006) Bullet fragments in deer remains: implications for lead exposure in avian scavengers. Wildl Soc Bull 34:167–170

Hunter B, Wobeser G (1980) Encephalopathy and peripheral neuropathy in lead-poisoned mallard ducks. Avian Dis 24:169–178

Jacobson E, Carpenter JW, Novilla M (1977) Suspected lead toxicosis in a bald eagle. JAVMA 171:952–954

Kaiser TE, Reichel WL, Locke LN, Cromartie E, Krynitsky AJ, Lamont TG, Mulhern BM, Prouty RM, Stafford CJ, Swineford DM (1980) Organochlorine pesticide, PCB, and PBB residues and necropsy data for bald eagles from 29 states: 1975–77. Pestic Monit J 13:145–149

Kelly A, Kelly S (2005) Are mute swans with elevated blood lead levels more likely to collide with overhead power lines? Waterbirds 28:331–334

Kenntner N, Tataruch F, Krone O (2001) Heavy metals in soft tissue of white-tailed eagles found dead or moribund in Germany and Austria from 1993 to 2000. Environ Toxicol Chem 20:1831–1837

Kéry M (2010) Introduction to winbugs for ecologists. Academic Press, Burlington

Kochert MN, Steenhof K (2002) Golden eagles in the U.S. and Canada: status, trends, and conservation challenges. J Raptor Res 36:32–40

Kochert MN, Steenhof K, Mcintyre CL, Craig EH (2002) Golden eagle (*Aquila chrysaetos*). In: Poole A (ed) The birds of North America online. Cornell Lab of Ornithology, Ithaca. http://bna.birds.cornell.edu/bna/species/684. doi:10.2173/bna.684. Accessed 9 May 2014

Kramer JL, Redig PT (1997) Sixteen years of lead poisoning in eagles, 1980–95: an epizootiologic view. J Raptor Res 31:327–332

Lasko TA, Bhagwat JG, Zou KH, Ohno-Machado L (2005) The use of receiver operating characteristic curves in biomedical informatics. J Biomed Inform 38:404–415

Locke LN, Thomas NJ (1996) Lead poisoning of waterfowl and raptors. In: Fairbrother A, Locke LN, Hoff GL (eds) Noninfectious diseases of wildlife, 2nd edn. Iowa State University Press, Ames, pp 108–117

Locke LN, Bagley GE, Frickie DN, Young LT (1969) Lead poisoning and aspergillosis in an Andean condor. JAVMA 155:1052–1056

MacDonald JW, Randall CJ, Ross HM, Moon GM, Ruthven AD (1983) Lead poisoning in captive birds of prey. Vet Rec 113(3):65–66

Mulhern BM, Reichel WL, Locke LN, Lamont TG, Belisle A, Cromartie E, Bagley GF, Prouty RM (1970) Organochlorine residues and autopsy data from bald eagles 1966-68. Pestic Monit J 4:141–144

Nadjafzadeh M, Hofer H, Krone O (2013) The link between feeding ecology and lead poisoning in white-tailed eagles. J Wildl Manag 77:48–57

Nelson TA, Mitchell C, Abbott C (1989) Lead-shot ingestion by bald eagles in western Arkansas. Southwest Nat 34:245–249

Neumann K (2009) Bald eagle lead poisoning in winter. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, pp 210–218

O'Halloran J, Myers AA, Duggan PF (1989) Some sub-lethal effects of lead on mute swan Cygnus olor. J Zool Lond 218:627–632

Pain DJ, Fisher IJ, Thomas VG (2009) A global update of lead poisoning in terrestrial birds from ammunition sources. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, pp 99–118

Pain DJ, Sears J, Newton I (1995) Lead concentrations in birds of prey in Britain. Environ Pollut 87:173–180

Pattee OH, Hennes SK (1983) Bald eagles and waterfowl: the lead shot connection. Trans N Am Wildl Nat Resour Conf 48:230–237

Pattee OH, Wiemeyer SN, Mulhern BM, Sileo L, Carpenter JW (1981) Experimental lead-shot poisoning in bald eagles. J Wildl Manag 45:806–810

Reichel WL, Schmeling SK, Cromartie E, Kaiser TE, Krynitsky AJ, Lamont TG, Mulhern BM, Prouty RM, Stafford CJ, Swineford DM (1984) Pesticide, PCB, and lead residues and necropsy data for bald eagles from 32 states: 1978-81. Environ Monit Assess 4:395–403

Rocke TE, Samuel MD (1991) Effects of lead shot ingestion on selected cells of the mallard immune system. J Wildl Dis 27:1–9

Rodriguez-Ramos Fernandez J, Höfle U, Mateo R, de Francisco ON, Abbott R, Acevedo P, Blanco JM (2011) Assessment of lead exposure in Spanish imperial eagle (Aquila adalberti) from spent ammunition in central Spain. Ecotoxicology 20:670–681

Route WT, Key RL, Russell RE, Lindstrom AB, Strynar MJ (2014) Spatial and temporal patterns in concentrations of perfluorinated compounds in bald eagle nestlings in the upper midwestern United States. Environ Sci Technol. doi:10.1021/es501055d

Russell RE, Franson JC (2014) Causes of mortality in eagles submitted to the National Wildlife Health Center 1975–2013. Wildl Soc Bull. doi:10.1002/wsb.469

Sabine N, Klimstra WD (1985) Ecology of bald eagles wintering in southern Illinois. Trans Ill Acad Sci 78:13–24

Saito K (2009) Lead poisoning of Steller's sea-eagle (Haliaeetus pelagicus) and white-tailed eagle (Haliaeetus albicilla) caused by the ingestion of lead bullets and slugs, in Hokkaido, Japan. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, pp 302–309

Smith JP, Farmer CJ, Hoffman SW, Kaltenecker GS, Woodruff KZ, Sherrington PF (2008) Trends in autumn counts of migratory raptors in western North America. In: Bildstein KL, Smith JP, Ruelas Inzunza E, Veit RR (eds) State of North America's Birds of Prey. Series in Ornithology 3. Nuttall Ornithological Club, Cambridge and American Ornithologists' Union, Washington DC, pp. 217–251

Stauber E, Finch N, Talcott PA, Gay JM (2010) Lead Poisoning of bald (Haliaeetus leucocephalus) and golden (Aquila chrysaetos) eagles in the US inland Pacific Northwest region—an 18-year retrospective study: 1991–2008. J Avian Med Surg 24:279–287

Sturtz S, Ligges U, Gelman A (2005) R2WinBUGS: a package for running WinBUGS from R. J Stat Softw 12:1–16

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/. Accessed 27 June 2014

Trust KA, Miller MW, Ringelman JK, Orme IM (1990) Effects of ingested lead on antibody production in mallards (Anas platyrhynchos). J Wildl Dis 26:316–322

Warner SE, Britton EE, Becker DN, Coffey MJ (2014) Bald eagle lead exposure in the upper Midwest. J Fish Wildl Manag 5:e1944-687X. doi:10.3996/032013-JFWM-029

Wayland M, Bollinger T (1999) Lead exposure and poisoning in bald eagles and golden eagles in the Canadian prairie provinces. Environ Pollut 104:341–350

Wayland M, Wilson LK, Elliott JE, Miller MJR, Bollinger T, McAdie M, Langelier K, Keating J, Froese JMW (2003) Mortality, morbidity, and lead poisoning of eagles in western Canada, 1986–98. J Raptor Res 37:8–18